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Frequent Expression of Complement Resistance Factors CD46, CD55, and CD59 on Gastrointestinal Cancer Cells Limits the Therapeutic Potential of Monoclonal Antibody 17-1A

HARTMUT JUHL, MD, PhD,* FRANK HELMIG, KATRIN BALTZER, HOLGER KALTHOFF, PhD, DORIS HENNE-BRUNS, MD, PhD, AND BERND KREMER, MD, PhD
Klinik für Allgemeine Chirurgie und Thoraxchirurgie, Christian-Albrechts Universität, Kiel, Germany

Background: One reason for the failure of monoclonal antibody (mab) trials in most cancer patients might be the presence of complement resistance factors that inhibit complement dependent cytotoxicity (CDC) and the release of inflammatory mediators (e.g., anaphylatoxins).
Method: We have determined the expression of CD46, CD55, and CD59 in five gastric, three colon, and seven pancreatic human cancer cell lines by immunostaining. The complement activating properties of mabs and conjugates with cobra venom factor (CVF) were studied in a ^{51}Cr-release toxicity assay and in an ELISA to determine the release of C3a.
Result: Virtually all tumor cell lines strongly expressed CD46, CD55, and CD59, except KATOIII gastric cancer cells (CD55 and CD59 negative). In accordance with other studies we could confirm that expression of CD55 and CD59 inhibits a complement activation by mabs. Whereas 17-1A was able to induce a cytotoxic complement activation on KATOIII cells, neither a CDC nor an anaphylatoxin release (C3a) was observed on MKN28 cells (strong expression of CD55 and CD59). Conjugation with CVF, a strong activator of the alternative pathway of complement, could partially restore the complement activation by mabs. A 17-1A-CVF conjugate, although still nontoxic, induced the release of the anaphylatoxin C3a on both cell lines. The same observations were made in PancTuI pancreatic cancer cells treated with a conjugate of the mab CA19-9 and CVF.
Conclusions: Our study shows that complement resistance is a frequent event in gastrointestinal cancer, limiting the potential of monoclonal antibodies. Mabs, when conjugated with CVF, partially retain complement activating properties by releasing C3a, which in vivo will support a cellular immune response. *J. Surg. Oncol. 64:222–230, 1997*

KEY WORDS: **complement resistance; monoclonal antibodies; adjuvant therapy; gastrointestinal cancer**

INTRODUCTION

The principal therapy of gastrointestinal cancers and pancreatic cancer is based on the surgical resection of the tumor and a radical lymphadenectomy. Chemotherapy and radiation in unresectable tumor stages have not shown a significant effect on survival. However, even

*Correspondence to: Hartmut Juhl, Clinic of General Surgery, University Hospital Kiel, Arnold-Heller Str., D-24105 Kiel, Germany
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patients with curative operable cancers (RO-resection) frequently suffer from an incurable relapse resulting in 5-year survival rates of 48% for gastric cancer [1], 81% for colon, 66% for rectal cancer [2], and 33% for pancreatic cancer [3]. Therefore, adjuvant therapy modalities that target postoperatively remaining tumor cells are of special interest. In Dukes C colorectal cancer, it was shown that a combination of 5-fluorouracil and levamisol [4] (in rectal cancer combined with local radiation [5]) positively affects the survival rate. Presently, in pancreatic [6] and in gastric cancer [7], no effective adjuvant therapy is available. In this situation new therapeutic concepts are urgently needed.

Since Köhler and Milstein developed the hybridoma technique in 1975 [8], many studies have been performed to target human malignancies with monoclonal antibodies (mabs). In vitro and in vivo experiments could achieve a tumor-specific toxicity by activating an antibody-dependent cellular cytotoxicity (ADCC) and/or a complement-dependent cytotoxicity (CDC) [9]. However, clinical phase I and II studies that used the monoclonal antibody 17-1A [10] showed no significant response rates in most patients with gastrointestinal cancer [11]. The poor results of antibody therapy trials are mainly due to a low antibody uptake in tumors of <0.1–0.01% of the injected dose, the antigen heterogeneity of solid carcinomas [12,13], and the expression of complement resistance factors CD46, CD55, and CD59, which can inhibit the activation of the complement cascade.

We focused our study on the last point because the activation of complement is an essential event in the humoral immune response. Inhibition of the complement cascade prevents tumor cells from cytolysis, which is caused by the formation of the factor C5b-C9 membrane attack complex (MAC) [14]. Furthermore, the anaphylatoxins C3a, C4a, and C5a support the cellular immune response by chemotactic attraction of phagocytes, increase of the capillary permeability and tumor vascularisation, and the induction of an interleukin-1 production by monocytes [15]. Finally, the lack of the complement component C3b and its inactivated form iC3b on the cell membrane of targeted cells (both initiate binding of macrophages, NK cells, and granulocytes via the C3-receptor) will further decrease the cytotoxic potential of the immune system [14].

CD46, CD55, and CD59 are the most important membrane-bound inhibitors of complement and react at various stages within the complement cascade. CD46 and CD55 block the complement cascade in an early stage and prevent anaphylatoxin release and the cytolysis by the MAC. Whereas CD46 (membrane cofactor protein) blocks the formation of a C3/C5 convertase by activating factor H and I [16], CD55 (decay-accelerating factor) dissociates the C3/C5 convertase independently from other proteins [17]. CD59 interacts in the last step of the complement cascade and prevents cytolysis by binding to C8 and thereby blocking of the C9-polymerization process [18].

Previous studies have shown that expression of CD55 and CD59 inhibits the ability of monoclonal antibodies to activate complement and thereby limits its therapeutic potential [18,19]. Therefore, we examined several gastrointestinal cancer cell lines for the expression of CD46, CD55, and CD59. We confirmed that the presence of CD55 and CD59 significantly reduces the ability of the monoclonal antibody 17-1A to activate complement.

The conjugation of Cobra Venom Factor (CVF) might improve the ability of mabs to activate complement [20]. CVF, a glycoprotein isolated from cobra venom (144 kDa), forms (analogous to human C3b) with factor Bb a C3/C5 convertase. The CVFBb complex—in contrast to C3bBb—cannot be inhibited by factor H and I and thereby permanently activates the alternative complement pathway [20]. We demonstrated in this study that a monoclonal antibody conjugated with CVF acquires complement activating properties on complement-resistant gastrointestinal cancer cells and can thereby improve antibody-directed therapy.

MATERIALS AND METHODS

The monoclonal antibody 17-1A [21] was purchased from Wellcome (Burgevetel, Germany). The mab CA19-9 was isolated from the supernatant of hybridoma culture by protein A affinity chromatography. The cancer cell lines and their characteristics are listed in Table I. Tumor cells were maintained in continuous culture using RPMI1640 medium with glutamine (Mediatech, Herndon), including 10% fetal calf serum (FCS) (GIBCO, Paisley, UK), penicillin (100 U/ml), and streptomycin (100 μg/ml).

CVF was isolated from lyophilized cobra venom (Naja naja kaouthia) (Latoxan, Rosans, France) as described previously [26]. Normal human serum was stored at −80°C and used within 2 weeks of preparation. Guinea pig serum and erythrocytes were obtained from the animal breeding colony of the University Hospital Eppendorf (Hamburg, Germany). N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP) and Sephadex-G200 Gel were purchased from Pharmacia (Piscataway, NJ), molecular weight markers for SDS polyacrylamide gel electrophoresis from Bio-Rad (Richmond, CA). Peroxidase and alkaline phosphatase labelled $F(ab')_2$ goat-antimouse antibodies for immunostaining and ELISA were purchased from Dianova. Antibodies against CD46, CD55, and CD59 were obtained from Serotec (Wiesbaden, Germany), the monoclonal antibody binding to C3a-complement factor was purchased from Quidel (San Diego, CA), and the $Na_2\ ^{51}CrO_4$ (1 mCi/ml) were obtained from Amersham (Arlington Heights, IL).

TABLE I. Origin and Histological Characterization of Human Cancer Cell Lines Used

Cell line	Histology	Origin
Gastric cancer		
KATOIII	low differentiated adenoca	[22]
MKN7	high differentiated adenoca	T. Suzuki, Niigata University, Japan
MKN28	high differentiated adenoca	T. Suzuki, Niigata University, Japan
MKN74	high differentiated adenoca	T. Suzuki, Niigata University, Japan
MKN45	low differentiated adenoca	T. Suzuki, Niigata University, Japan
Colorectal cancer		
HT 29	moderately differentiated adenoca	[23]
SW 1116	moderately differentiated adenoca	[24]
WiDr	moderately differentiated adenoca	ATCC, Rockville, MA
Pancreatic cancer		
ASPC-1	high differentiated adenoca	ATCC, Rockville, MA
Capan-2	high differentiated adenoca	[23]
Colo357	high differentiated adenoca	[25]
PancTuI	moderately differentiated adenoca	v. Bülow, University Mainz, Germany
PancTuII	low differentiated adenoca	v. Bülow, University Mainz, Germany
SW 850	low differentiated adenoca	[23]
SW 979	low differentiated adenoca	[23]

Immunocytochemistry

Immunostaining of human cancer cells was performed by APAAP-staining using the staining system and method obtained from Dianova. The evaluation of microscopic slides included the intensity of staining (− = no reaction, + = low reaction, ++ = medium strong reaction, +++ = very strong reaction) and the estimated number of positive cells (% of positive cells).

ELISA Binding Assay

The binding properties of the antibodies and CVF conjugates were determined by ELISA technique. Tumor cells (10^5 cells/well) were plated on microtiter plates until confluent, fixed with 0.05% glutaraldehyde, and blocked at 4°C with 1% BSA in PBS overnight. The plates were washed and antibodies were added in varying amounts (100 μl/well) and incubated for 90 min/20°C. Cells were washed extensively (3×) and then incubated with a goat peroxidase-conjugated antimouse IgG (Dianova) diluted 1:30,000 in PBS, pH 7.45 (100 μl/well) for 1 hour at 20°C. After washing, cells were incubated with 100 μl/well substrate (4 mg o-phenylenediamine in 10 ml 0.1 M citrate-disodiumphosphate buffer pH 5.0 containing 3.3 μl 30% H_2O_2) for 20 min at 20°C. The reaction was blocked by adding 8 N H_2SO_4 (25 μl/well) and absorbance was determined at 490 nm.

Binding of 17-1A to Living Cells (FACS-analysis)

To determine the binding of 17-1A antibody to living cells, FACS-analysis was used; 10^6 MKN28 and KATOIII cells, respectively, were incubated for 20 min. at 4°C with mouse serum (diluted 1:25 in culture medium), washed, and 100 μl of mab 17-1A (0.5–50 μg/ml) were added. After 30 min at 37°C, the cells were washed three times with medium and mixed with 100 μl (3 μg/ml) of a FITC-labelled goat $F(ab')_2$ antimouse IgG antibody (30 min at 4°C). Cells were 3× washed extensively with medium, resuspended with 0.5 ml culture medium, and cell clots were removed by filtration. The fluorescence intensity of 5×10^3 cells was measured and the amount of antibody binding cells was determined with a Becton Dickenson FAC StarPLUS flowcytometer/cell sorter.

^{51}Cr Release Cytotoxicity Assay

Tumor cells were labelled with $Na_2{}^{51}CrO_4$ to a specific activity of 0.1–0.2 cpm/cell as described [27]. The labeled cells (5×10^4) were incubated for 45 min at 37°C with varying amounts of either unconjugated antibodies, CVF conjugates, or free CVF in a total volume of 100 μl medium. The cells were washed, resuspended with human serum 1:2 diluted in PBS, pH 7.45, and the cell culture cluster microtiter plates (Costar, Cambridge, MA) were coated with 10^4 cells/well. After 4 hours at 37°C, the supernatant was collected and radioactivity was measured using a gamma counter. Culture medium was used to determine background activity and maximal ^{51}Cr-release was measured by the addition of 10% (w/v) SDS instead of serum. Specific ^{51}Cr release was calculated as: 100× [(experimental cpm-background cpm)]: [maximal cpm-background cpm]. Experiments were performed in triplicate.

Preparation of mab-CVF Conjugate

The conjugation of mab 17-1A and CA19-9 with CVF was performed as described previously [26]. Briefly, 6 mg mab were derivatized with SPDP (400 μmol). After purification by size exclusion chromatography using G-25 Sephadex PD10 columns from Pharmacia, the antibody fractions were concentrated by ultrafiltration tubes (Sartorius, Göttingen, Germany) and incubated with 50 mM dithiothreitol (DTT). After 20 min, DTT

was removed and the free sulfhydryl-containing antibodies were immediately added to equimolar amounts of SPDP-derivatized CVF (4 mg). The derivatization with SPDP yielded 3.5 pyridyldithio residues per CVF and three residues per antibody molecule, respectively. After 22 hours, the reaction mixtures were subjected to size exclusion chromatography at 4°C on a Sephadex-G200 gel column (2.5 × 100 cm) equilibrated with PBS, pH 7.45 (GIBCO). The conjugate fractions were pooled and concentrated by ultrafiltration tubes (Sartorius) to a final concentration of 200 μg/ml mab-CVF conjugate. After filter sterilization, the conjugate was found to be stable for at least several months at 4°C. All calculations are based on a molecular weight of 155,000 for the antibody and 299,000 for the conjugate with CVF (MW 144,000). Determination of the hemolytic activity and antigen binding capability is based on the assumption of an equimolar ratio of CVF and antibody in conjugates.

Protein concentrations were determined spectrophotometrically at 280 nm using extinction coefficient of $E^{0.1\%} = 0.99$ for CVF [26], $E^{0.1\%} = 1.4$ for antibodies and $E^{0.1\%} = 1.2$ for the mab-CVF conjugates.

Hemolytic Assay for CVF Activity

The complement-activating properties of free and conjugated CVF were determined in a bystander lysis assay of guinea pig erythrocytes [27]. Briefly, unsensitized guinea pig erythrocytes (5×10^8 cells/20 μl) were incubated for 30 min at 37°C with 20 μl of guinea pig serum and 20 μl of varying amounts of mab-CVF or unconjugated proteins in PBS, pH 7.45. Hemolysis was determined by spectrophotometric quantitation of released hemoglobin at 412 nm.

ELISA for Detection of C3a Release

To determine a release of the anaphylatoxin C3a, we used an ELISA test system with a mouse anti-C3a monoclonal antibody (Quidel), which was proven specifically to detect C3a as described previously [28]. Briefly, 10^6 cells were incubated with varying amounts of antibody, conjugate or free CVF, respectively. After 90 min at 20°C on a shaker, the cells were washed three times with culture medium and were incubated for 90 min 20°C, with 1 ml human serum. Cells were centrifuged (1,000 rpm/5 min) and C3a (MW 8,000) was separated from intact C3 by ultrafilter-centrifugation (2,500 rpm/45 min) with centrisat-tubes separating molecules MW < 10,000 (Sartorius). The eluent was diluted 1:20 with PBS, pH 7.45, and 100 μl/well were pipetted into microtiter plates and incubated for 2 hours at 20°C. Plates were washed three times with 1.5% BSA in PBS, pH 7.45 and blocked overnight at 4°C with 300 μl/well of 1% BSA in PBS, pH 7.45. After washing, the plates were incubated with 100 μl/well of the mouse anti-C3a antibody, diluted 1: 40,000 (1% BSA in PBS, pH 7.45). After 2 hours at 20°C, the plates were washed and 100 μl/well of the peroxidase labeled antimouse IgG antibody was added (60 min/20°C). Finally, cells were incubated with 100 μl/well of substrate (10 ml 0.1 M citrate-disodium-phosphate buffer, pH 5.0 containing 4 mg o-phenyldiamine and 3.3 μl 30% H_2O_2) at 20°C. After 30 min, the reaction was stopped by the addition of 8 N H_2SO_4 (20 μl/well) and absorbance was measured in a microelisa auto reader at 490 nm.

RESULTS

Expression of CD46, CD55, and CD59

CD46 was detected on all five gastric, three colorectal, and seven pancreatic cancer cell lines. CD55 could be found on 4/5 gastric cancer cell lines (no staining on KATOIII cells), 3/3 colorectal, and 7/7 pancreatic cancer cell lines. One pancreatic cancer cell line (PancTuII) expressed CD55 only with low intensity on 5% of the tumor cells; all other positive cell lines showed a medium strong to very strong reaction on 50–95% of the cells. CD59 was detected on virtually all cell lines and in the majority of cells, except one gastric cancer cell line (KATOIII), which expressed CD59 only on 5% of the cells with low intensity. The results are summarized in Table II.

Binding of 17-1A to MKN28 and KATOIII Gastric Cancer Cells

To confirm that expression of CD55 and CD59 leads to an inhibition of complement, cell lines were identified that bind 17-1A to a comparable degree but differ in their resistance factor expression. Approximately 80% of MKN28 cells and 65% of KATOIII cells, determined by ELISA technique and FACS analysis, bound the mab 17-1A (Fig. 1). MKN28 cells, in contrast to KATOIII cells, strongly expressed CD55 and CD59. These cell lines were choosen to correlate the complement-mediated effects of 17-1A antibody with the expression of complement resistance factors.

Cytotoxicity of 17-1A Against KATOIII and MKN28 cells

MKN28 and KATOIII cells were incubated with varying amounts of 17-1A antibody and human serum. The complement mediated toxicity was measured in a ^{51}Cr-release assay. In three independently performed assays, it was found that 50% to maximal 80% of KATOIII cells could be eliminated by complement, whereas no toxicity was observed with MKN28 cells (Fig. 2).

Release of Anaphylatoxin C3a by 17-1A Treatment

KATOIII and MKN28 cells were treated with 17-1A antibody/human serum and the release of C3a was determined by the ELISA technique. The treatment of MKN28 cells did not result in a C3a release, whereas

TABLE II. Results of APAAP Staining for Complement Resistance Factors CD46, CD55, and CD59*

Cell line	CD46	CD55	CD59
Gastric cancer			
KATOIII	++ (100%)	− (0%)	+ (5%)
MKN28	++ (75%)	++ (75%)	++ (75%)
MKN7	++ (75%)	++ (50%)	++ (75%)
MKN45	+++ (95%)	+++ (95%)	+++ (95%)
MKN74	+++ (75%)	++ (75%)	+++ (75%)
Colorectal cancer			
HT29	+++ (75%)	++ (75%)	++ (75%)
SW 1116	++ (75%)	++ (75%)	++ (75%)
WiDr	++ (75%)	++ (75%)	++ (75%)
Pancreatic cancer			
ASPC-1	++ (95%)	++ (95%)	++ (75%)
Capan-2	++ (75%)	++ (75%)	++ (75%)
Colo357	+ (50%)	++ (75%)	++ (50%)
PancTuI	++ (50%)	++ (75%)	++ (75%)
PancTuII	+ (50%)	+ (5%)	++ (25%)
SW 850	++ (75%)	++ (75%)	++ (75%)
SW 979	+++ (100%)	++ (95%)	+++ (100%)

*Microscopic slides were evaluated for the intensity of staining (− = no reaction, + = low reaction, ++ = medium strong reaction, +++ = very strong reaction) and the estimated number of positive cells (% of positive cells).

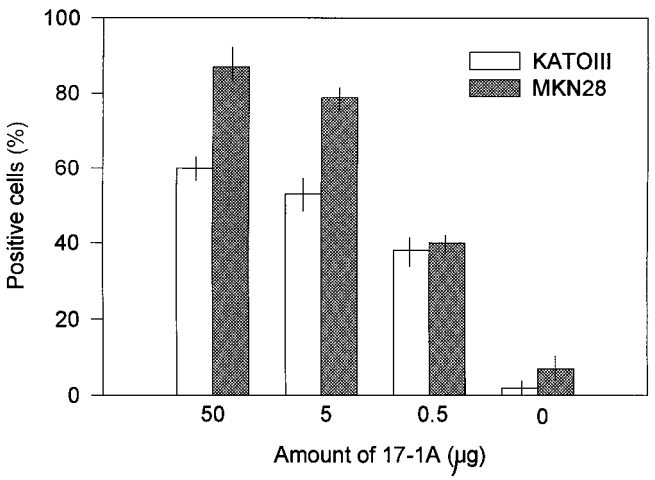

Fig. 1. FACS-analysis was used to calculate the amount of antibody reactive tumor cells in correlation to the applied 17-1A amount.

17-1A induced a significant production of C3a on KATOIII cells (Fig. 3).

Synthesis and Characterization of 17-1A-CVF Conjugate

The purity of the 17-1A-CVF conjugate was tested by a 10% SDS-polyacrylamide gel electrophoresis and showed virtually no uncoupled protein. The conjugate fraction was seen at a molecular weight level of 300,000–500,000 indicating a composition of primarily di- and trimers (data not shown). The binding activity, determined with MKN28 cells, showed no difference between

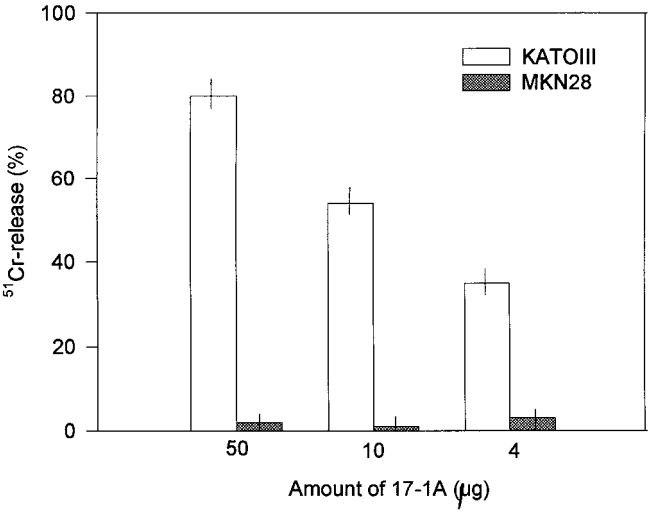

Fig. 2. The complement mediated cytotoxicity of 17-1A antibody was determined by measuring the ^{51}Cr-release of labelled KATOIII and MKN28 cells using varying amounts of antibody and human serum as complement resource.

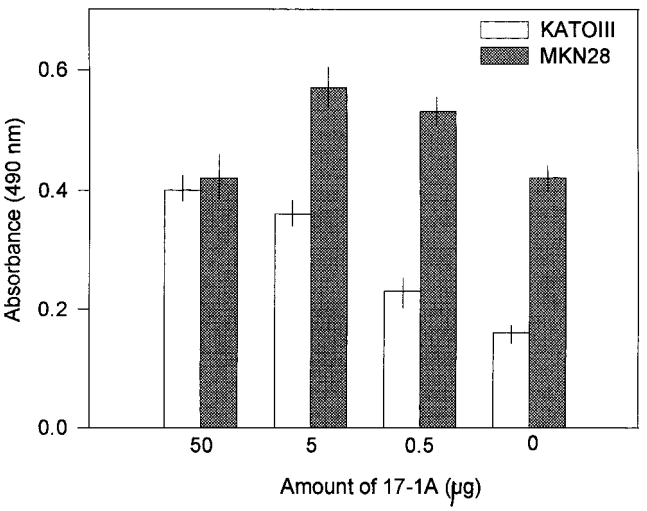

Fig. 3. The relative amount of C3a in the supernatant of KATOIII and MKN28 cells, treated with varying amounts of 17-1A antibody is shown (ELISA technique).

binding of 17-1A antibody and its CVF-conjugate (Fig. 4a). Using a hemolytic bystander assay, the conjugate contained complement-activating properties that were reduced by 40% compared to noncoupled CVF (Fig. 4b).

17-1A-CVF Effect Against KATOIII and MKN28 Cells

17-1A-CVF did not express a cytotoxic effect against MKN28 cells as it was determined by a ^{51}Cr-release assay (data not shown). However, treatment of MKN28 and KATOIII cells with the 17-1A-CVF conjugate and determination of C3a production by ELISA technique resulted—in contrast to the noncoupled antibody—in a

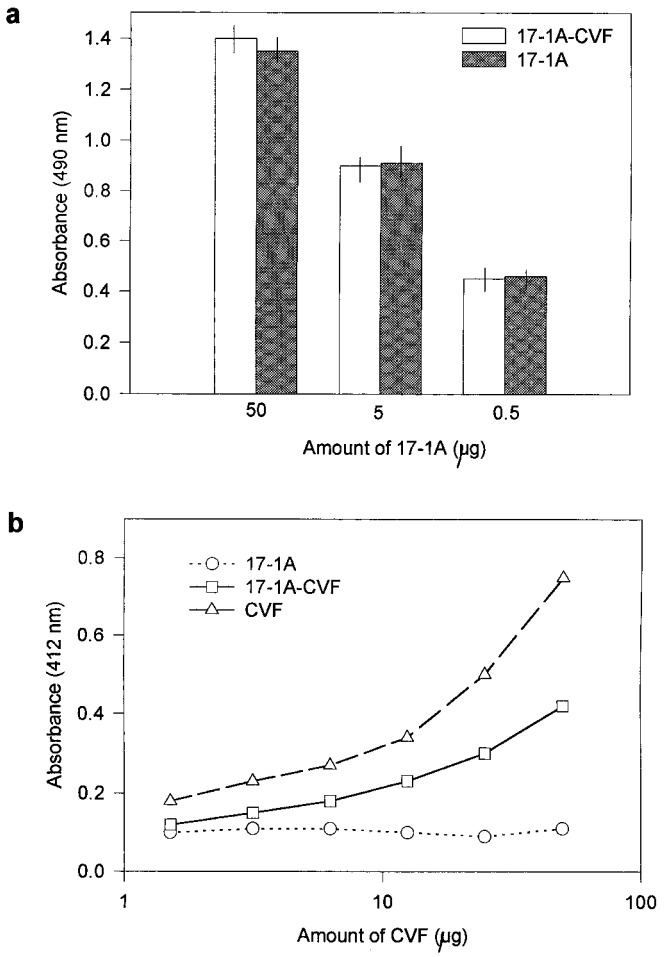

Fig. 4. The binding activity of 17-1A-CVF conjugate was compared with noncoupled antibody by ELISA-technique using MKN28 cells **(a).** The complement activating properties of antibody bound CVF was measured in a hemolytic assay using guinea pig erythrocytes and serum and compared with noncoupled CVF **(b).**

significant release of C3a on both cell lines (Fig. 5). Furthermore, the values of C3a release on KATOIII cells exceeded the results of 17-1A.

Ability of a CA19-9-CVF Conjugate to Induce Complement Activation on Pancreatic Cancer Cells

To prove further that conjugation of CVF can partially restore the complement activation on resistance factor positive cells, a different cell system using a different mab was investigated. The pancreatic cancer cell line PancTuI strongly expressed CD46, CD55, and CD59. The monoclonal antibody CA19-9, which binds to PancTuI cells, was coupled with CVF. No uncoupled protein was seen in 10% SDS-polyacrylamid gel electrophoresis. Determined by the ELISA-technique, the binding activity of the CA19-9 antibody was not reduced by conjugation of CVF (Fig. 6a). In a hemolytic bystander assay the complement-activating properties of the CA19-9-CVF conjugate were shown (Fig. 6b). Complement-mediated

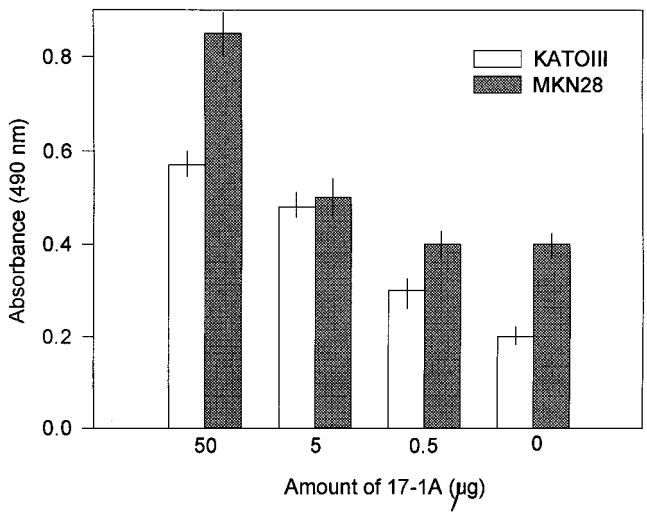

Fig. 5. KATOIII and MKN28 cells were treated with 17-1A-CVF and the C3a release was determined by ELISA technique.

cell lysis could not be achieved by CA19-9 or by CA19-9-CVF conjugate (data not shown). However, the CA19-9-CVF conjugate induced a significant release of C3a on CD55 and CD59 positive PancTuI cells (Fig. 7).

DISCUSSION

The main problem in the oncological therapy of gastrointestinal cancers is local relapse and the occurrence of metastases following an apparently curative tumor resection. Therefore, new adjuvant therapy concepts are under investigation that target postoperatively remaining tumor cells. Only in colorectal cancer could a chemotherapeutic approach with 5-fluorouracil and levamisole be proven to increase the survival rate in Dukes C tumor stages [4]. So far, in gastric and pancreatic cancer no significant effects of any adjuvant chemotherapy have been observed [6,7]. Drug resistance and the lack of a functioning p53 tumor suppressor gene, which mediates a chemo- and radiotherapeutic effect by inducing apoptosis, are several reasons for the failure of chemotherapy [29]. Disseminated single tumor cells are already detectable in peritoneal cavity and the bone marrow even in early tumor stages of gastrointestinal cancers by immunocytology [30]. They are most likely a source for metastatic tumor progress and not efficiently treatable by cytostatic drugs due to their dormant state [31].

In this situation, monoclonal antibodies (mabs) are of special interest. They offer the chance for a highly specific treatment without severe side effects and may also target dormant cells by ADCC or CDC. Most clinical antibody studies were performed with patients in an advanced tumor stage and did not show a significant response [9]. This inefficiency is mainly due to a low tumor specific uptake, caused by a high interstitial pressure that further reduces the slow diffusion of the large mol-

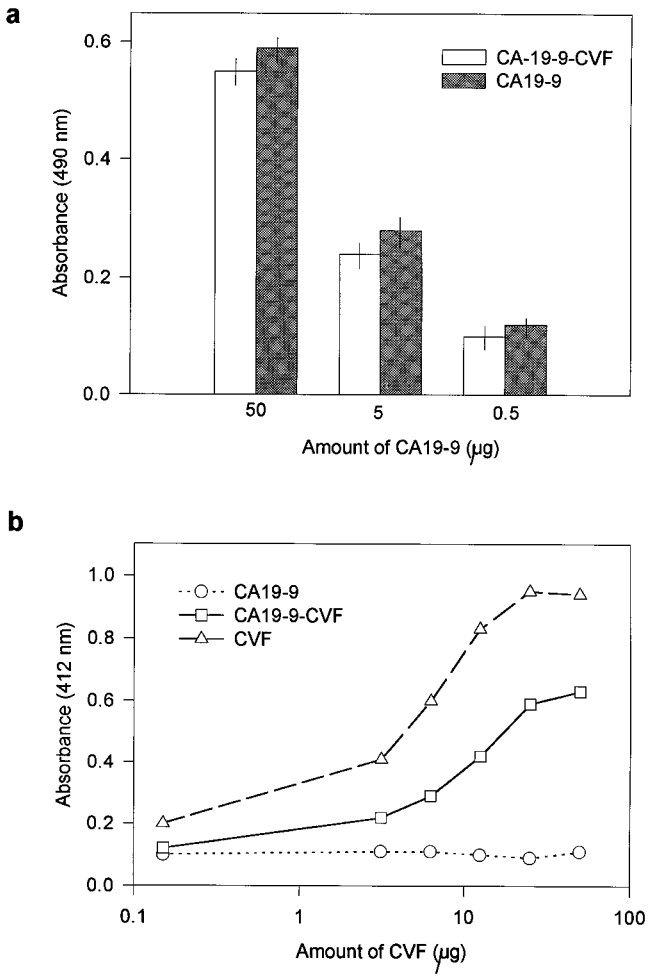

Fig. 6. The binding activity of CA19-9-CVF conjugate was compared with noncoupled antibody by ELISA-technique using PancTuI cells **(a).** The complement activating properties of conjugated CVF was measured in a hemolytic assay using guinea pig erythrocytes and serum and compared with noncoupled CVF **(b).**

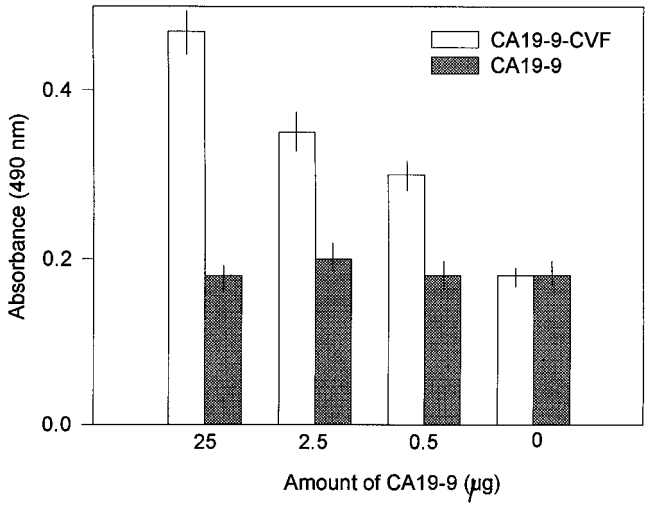

Fig. 7. The relative amount of C3a in the supernatant of PancTuI cells, treated with varying amounts of CA19-9 antibody and CA19-9-CVF conjugate, is shown (ELISA technique).

ecules into the tumor tissue [13]. This problem is less important in adjuvant therapy approaches because antibodies target only small tumors and thereby reach most tumor cells more easily by diffusion. The potential of cytotoxic antibodies was shown in a clinical trial with patients suffering from a Dukes C colon carcinoma. Following a curative tumor resection, they were treated with the mab 17-1A and showed a significant better survival rate than untreated patients [32]. However, other obstacles remain and may explain why 17-1A still failed to prevent metastases in the majority of treated patients.

Complement supports the cellular cytotoxicity of antibodies (ADCC) by enhancing the vascular permeability, blood flow, and especially chemotaxis of phagocytes by anaphylatoxins. Furthermore, it improves the binding of phagocytic cells to tumor cells via an opsonizing reaction of C3b and iC3b [14]. Subsequently, the presence of complement resistance factors, which inhibit an antibody mediated complement activation, might be one important factor for the failure of antibody tumor therapy.

Another problem in clinical trials is the antigen heterogeneity of tumors containing cell subpopulations that apparently differ in their antigen expression and that may be solved by applying a panel of different mabs covering the variations of antigen expression within the tumor [33].

The significance of complement resistance of gastrointestinal cancer cells has not been studied extensively. Using immunostaining, we detected CD46, CD55, and CD59 virtually on all tested human gastric, colorectal, and pancreatic cancer cell lines. This high frequency is in accordance with findings in lung carcinoma. CD46 and CD59 was expressed on all tested 25 cell lines and CD55 on 24/25 cell lines [34]. Interestingly, the expression of complement resistance factors in cancer cells seems to be a rather specific event of malignant cells. CD55 was detected in 67% of colon cancer tissue samples, whereas normal mucosa showed CD55 only sporadically [35].

In our study, only one cancer cell line, the gastric cancer cell line KATOIII, did not express CD55 (decay accelerating factor), which dissociates the C3/C5 convertase and thereby inhibits the complement cascade completely [17]. Additionally, KATOIII cells were only to a small extent positive for the inhibitor of the membrane attack complex (MAC), CD59. We could show that 17-1A antibody induces on the CD55 and CD59 negative cell line KATOIII, a complement-mediated cell lysis of 50–80% and a release of the anaphylatoxin C3a. The antibody could neither achieve a cytotoxic effect on CD55 and CD59 positive MKN28 gastric cancer cells nor cause a release of inflammatory mediators (C3a). This observation is in accordance with several studies, which showed that expression of CD55 and CD59 inhib-

its an antibody-mediated complement activation [18,19] and thereby hinders a comprehensive activation of the immune system by antibodies.

We examined whether complement activation in resistant cells is achievable by coupling Cobra Venom Factor (CVF) to 17-1A. CVF, isolated from cobra venom, is a nontoxic glycoprotein that permanently activates the alternative pathway, due to the formation of a stable CVFBb complex [20]. Several studies have shown that mab-CVF conjugates can efficiently lyse melanoma [27], leukemia [36], and neuroblastoma cells [37] via complement activation. We could show that a 17-1A-CVF conjugate is able to activate the complement cascade and to release the anaphylatoxin C3a on gastrointestinal cancer cells despite their expression of CD46, CD55, and CD59.

To confirm that these findings are of general value in gastrointestinal cancer, we investigated the pancreatic cancer cell line PancTuI, which strongly expressed CD46, CD55, and CD59. In contrast to uncoupled antibody, the conjugate of CA19-9-CVF showed on PancTuI cells the same characteristics as did the 17-1A-CVF conjugate on MKN28 cells. Although nontoxic, CA19-9-CVF achieved a significant release of the anaphylatoxin C3a.

Antibodies are able to eliminate tumor cells by ADCC and independent from complement. However, the synergistic effect of complement and ADCC within the immune system strongly suggests that the introduction of complement-activating properties to a mab will improve the therapeutic results. The potential of a mab-CVF conjugate in treating complement resistance factor positive tumors has been shown in an orthotopic human pancreatic cancer model using nude rats. A mab-CVF conjugate was able to induce an inflammatory reaction within the tumor and significantly increased the tumor infiltration by NK cells and macrophages [28]. However, although CVF is a fairly nontoxic component [38–40], its clinical use will be limited by its strong immunogenicity [41]. Therefore, the comparison of the DNA sequence with its human analogue C3 is currently under investigation with the aim to manufacture a manipulated human C3 that possesses the permanent complement-activating properties of CVF (C.-W. Vogel, Dept. of Biochemistry and Molecular Biology, University Hamburg, Germany, pers. comm.).

Recently, a recombinant 17-1A antibody was produced that contained a murine binding region and an ADCC mediating human antibody Fc-fragment [42]. The production of fusion proteins that possess not only tumor binding properties and ADCC effector function, but also properties of a CVF molecule, might help increase overall antitumor response and therefore improve the results of antibody-directed adjuvant therapy.

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